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(54) Title: AN IMMUNOTOXIN WITH IN VIVO T CELL SUPPRESSANT ACTIVITY

(57) Abstract

The present invention relates to an immunotoxin. The invention further relates to a method of treating T cell leukemias and lymphomas, graft-versus-host diseases, and autoimmune diseases by administering an immunotoxin.

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AN IMMUNOTOXIN WITH IN VIVO T CELL SUPPRESSANT ACTIVITY
Background of The Invention
Field of the Invention

The present invention relates to an immunotoxin.
5 The invention further relates to a method of treating T
cell leukemias or lymphomas, graft-versus-host diseases,
and autoimmune diseases by administering an immunotoxin.

Background Information

Immunotoxins are toxins with altered receptor
10 specificities. The alteration is achieved by coupling a
monoclonal antibody (mAb) or growth factor to the toxin
or toxin fragment. Plant and bacterial protein toxins
intoxicate cells by a multi-step process whereby
different toxin domains sequentially interact with
15 cellular components. The intoxication pathway at a
minimum consists of surface receptor binding, toxin
processing, intracellular routing of toxin A chains to
the cytosol, and enzymatic inactivation of protein
synthesis (Neville and Hudson (1986) Ann. Rev. Biochem.
20 55:195). The goal of immunotoxin research has been to
achieve targeted cell killing comparable to the enormous
but indiscriminate cell killing power of the native
toxins. An equally important goal has been to maintain
the low non-target cell toxicity of toxin A chains, which
25 lack cell receptor binding and membrane translocation
functions (Youle and Neville (1982) J. Biol. Chem.
257:5098; Neville (1986) in CRC Crit. Rev. Therap. Drug
Carrier Syst., CRC Press Inc. 2:329; Immunotoxins,
Frankel ed. (188) Kluwer Academic Publishers). Because
30 of this latter consideration most in vivo clinical
studies have focused on A chain immunotoxins or
immunotoxins with truncated B chains lacking the receptor
binding domain. While some clinical results have been
encouraging, the reproducible achievement of both goals

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is at present uncertain (Program and Abstracts 2nd Int. Symposium on Immunotoxins, June 1990, Lake Buena Vista, Florida).

   Recently, Youle and co-workers have introduced
5 highly efficacious holo-immunotoxins based on Diphtheria toxin (DT) binding mutants (Greenfield et al (1987) Science 238:536; Johnson et al. (1988) J. Biol. Chem. 263:1295; Johnson et al J. Neurosurg. 70:240). These DT binding site mutants were equal to the wild type
10 immunotoxins in potency when directed at the human transferrin receptor (TFR) or human CD3, a component of the T cell receptor complex. Since the binding of the mutants was only 1/100-1/1000 of native DT, the toxin receptor appeared to be not needed along the intoxication
15 pathway. This conclusion is limited to CD3 and TFR directed immunotoxins because similar immunotoxins directed at CD5 and the high-molecular weight-melanoma-associated antigen are relatively non-toxic (Neville et al. (1989) J. Biol. Chem. 264:14653). On the basis of
20 data obtained with acid-cleavable conjugates which released free DT or the DT binding site mutant CRM9 in acidified endosomes, it was concluded that the DT receptor participates in the optimal intracellular routing of DT and many DT conjugates (Neville et al
25 (1989) J. Biol. Chem. 264:14653). It was also concluded that CD3 and TFR can perform the same routing function as the DT receptor, thus obviating the requirement of a DT receptor interaction for the binding site mutant conjugates anti-CD3-CRM9 and TF-CRM9 (Intracellular
30 routing of ricin based immunotoxins via the ricin receptor leading to enhanced efficacy has also been reported. Youle et al. (1981) Cell 23:551; Marsh and Neville (1986) Biochem. 25:4461; Youle and Colombatti

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(1987) J. Biol. Chem 262:4676).   Since anti-CD3-CRM9
appears to achieve optimal routing with low non-target
cell toxicity as judged by _in vitro_ assays, the present
invention relates to a method of eradicating human CD3
5   bearing tumors _in vivo_.

        The present invention provides in one embodiment,
the immunotoxin anti-CD3-CRM9.   The invention provides,
in further embodiments, methods of treating T cell
leukemias or lymphomas, graft-versus-host diseases, and
10 autoimmune diseases by administering the immunotoxin
anti-CD3-CRM9.

Summary of the Invention

        It is a general object of this invention to provide
an immunotoxin.

15   It is a specific object of this invention to provide
an immunotoxin corresponding to anti-CD3-CRM9 or
derivatives thereof.

        It is a further object of the invention to provide
a method of treating T cell leukemias or lymphomas which
20 carry the CD3 epitope.

        It is another object of the invention to provide a
method of treating graft-versus-host disease.

        It is a further object of the invention to provide
a method of treating autoimmune diseases.

25   Further objects and advantages of the present
invention will be clear from the description that
follows.

        In one embodiment, the present invention relates to
an immunotoxin comprising anti-CD3-CRM9 or derivatives
30 thereof.

        In another embodiment, the present invention relates
to a pharmaceutical composition comprising anti-CD3-CRM9
or derivatives thereof in an amount effective to treat T

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cell leukemias or lymphomas which carry the CD3 epitope, graft-versus-host disease or autoimmune diseases, and a pharmaceutically acceptable diluent, carrier, or excipient.

5   In a further embodiment, the present invention relates to a method of treating T cell leukemias or lymphomas which carry the CD3 epitope in an animal comprising administering to an animal anti-CD3-CRM9 or derivatives thereof under conditions such that the
10 leukemias or lymphomas regress.

In another embodiment, the present invention relates to a method of treating graft-versus-host disease in an animal comprising administering to an animal anti-CD3-CRM9 or derivatives thereof under conditions such that
15 the symptoms of the graft-versus-host disease improve.

In a further embodiment, the present invention relates to a method of treating autoimmune diseases in an animal comprising administering to the animal anti-CD3-CRM9 or derivatives thereof under conditions such that
20 the symptoms of the autoimmune disease improve. In one preferred embodiment, the autoimmune disease is AIDS.

Brief Description of the Drawings

Figure 1. Nude mice bg/nu/xid maintained in a semi-sterile environment are preconditioned with 400 cGy whole
25 body ^{137}Cs γ radiation on day -7. On day 0, 2.5 x 10^7 Jurkat cells (human T cells leukemia CD3+, CD4+, CD5+) are injected subcutaneously with 1X10^7 HT-1080 feeder cells (human sarcoma) which have received 6000 cGy. Jurkat cells were passaged every other week in mice as
30 subcutaneous tumors and dissociated by collagenase/dispase prior to inoculation. This cell population exhibits a 40% inhibition of protein synthesis after 5 hours exposure to 10^{-11}M anti-CD3-DT. Clones

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isolated from this population by infinite dilution
exhibit varying sensitivity to anti-CD3-DT (4 less
sensitive, 3 more sensitive) corresponding to a 1.5 long
variation in dose response curves. Immunotoxin treatment
5   is given by intraperitoneal injection starting on day 7
when the tumor is visibly established. Evaluation takes
place on day 37.

Detailed Description of the Invention

The present invention relates to an immunotoxin.

10   In one embodiment, the present invention relates to
an immunotoxin comprising anti-CD3-CRM9 or derivatives
thereof. The design of successful derivatives of anti-
CD3-CRM9 depend upon understanding how the unique
concentration of anti-CD3-CRM9 achieves its biological

15   effect. The toxin moiety CRM9 retains its toxic
function, and membrane translocation function to the
cytosol in full amounts. The loss in binding function
located in the C terminus of the protein diminishes
systemic toxicity by reducing binding to non-target

20   cells. The routing function normally supplied by the
toxin binding function is supplied by the targeting
antibody anti-CD3. The essential routing pathway is (1)
localization to coated pits for endocytosis, (2) escape
from lysosomal routing, and (3) return to the plasma

25   membrane. Any antibody which can route in this manner
will be effective with CRM9, irrespective of which
epitope the antibody is directed. Thus, a wide variety
of cell types can in principle be targeted. When
antibodies dissociate from their receptors due to changes

30   in receptor configuration induced in certain receptors as
a consequence of endosomal acidification, they enter the
lysosomal pathway. This can be prevented or minimized by
directing the antibody towards an ecto-domain epitope on

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the same receptor which is closer to the plasma membranes
(Ruud, et al (1989) Scand. J. Immunol. 29:299; Herz et al
(1990) J. Biol. Chem. 265:21355). Other DT binding site
mutants can be used to form derivatives by changing amino
5 acids in the C terminus which can reduce the binding
function as long as the translocation function is
maintained.

An example of a series of derivatives which is
likely to be effective are antibody-CRM9 conjugates
10 directed at unique $V\alpha$ and $V\beta$ gene segment products of the
T cell receptor. Some of these epitopes appear to be
biased towards specific autoimmune processes. Such
conjugates should be useful in specific autoimmune
diseases (Kappler et al (1987) Cell 49:263; Urban et al
15 (1988) Cell 54:577).

Both acid-cleavable and non-cleavable protein cross-
linking reagents can be used in the construction of
antibody-diphtheria toxin binding-site mutant conjugates
like anti-CD3-CRM9 (Neville et al (1989) J. of Biol.
20 Chem. 264:14653-14661); preferred are non-cleavable
crosslinkers, such as bismaleimidohexane and
m-maleimidobenzoyl-N-hydroxysuccinimide ester. The
synthesis of acid-cleavable protein cross-linking
reagents based on orthoester, acetal, and ketal
25 functionalities has been described (Srinivasachar and
Neville (1989) Biochemistery 28:2501-2509). The unique
feature of these functionalities is that their observed
hydrolytic rate constants increase 10-fold for each drop
in pH, a consequence of specific H_3O^+ catalysis leading to
30 a carbonium ion intermediate (Cordes and Bull (1974)
Chem. Rev. 74:581-603). Moreover, these functionalities
are resistant to base catalysis permitting manipulation
and storage at alkaline pH. The cross-linking reagents

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react with proteins via heterobifunctional groups (maleimide and N-hydroxysuccinimide ester) of homobifunctional groups (bis-maleimide). The maleimide cross-linking is accomplished by prior protein thiolation 5 with iminothiolane. Cross-linked proteins exhibit first-order dissociation under acid conditions. The $t_{\frac{1}{2}}$ at pH 5.5 varies between 0.1 and 130 h for a series of six different cleavable cross-linkers (Srinivasachar and Neville (1989) Biochemistry 28:2501-2509).

10   In another embodiment, the present invention relates to a pharmaceutical composition comprising anti-CD3-CRM9 or derivatives thereof in an amount effective to treat T cell leukemias or lymphomas which carry the CD3 epitope, graft-versus-host disease or autoimmune diseases, and a 15 pharmaceutically acceptable diluent, carrier, or excipient. One skilled in the art will appreciate that the amounts to be administered for any particular treatment protocol can readily be determined. Suitable amounts might be expected to fall within the range of 7.5 20 to 75 µg per kg of body weight.

      In a further embodiment, the present invention relates to a method of treating T cell leukemias or lymphomas which carry the CD3 epitope in an animal comprising administering to an animal anti-CD3-CRM9 or 25 derivatives thereof under conditions such that the leukemias or lymphomas regress. Appropriate concentrations and dosage unit sizes can be readily determined by one skilled in the art.

      In another embodiment, the invention relates to a 30 method of treating an immune system disorder not involving T cell proliferation which is emenable to T cell suppression. In a specific embodiment, the present invention relates to a method of treating graft-versus-

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host disease (GVHD) in an animal comprising administering
to an animal anti-CD3-CRM9 or derivatives thereof under
conditions such that the symptoms of the graft-versus-
host disease improve. GVHD is a morbid complication of
5 bone marrow transplantation which is often performed as
anti-leukemia/lymphoma therapy.   GVHD is caused by
circulating donor T cells within the host which are
acquired in bone marrow grafts unless specifically
depleted prior to grafting (Gale and Butturini (1988)
10 Bone Marrow Transplant 3:185; Devergie et al. (1990) ibid
5:379; Filipovich et al. (1987) Transplantation 44).
Successful donor T cell depletion techniques have been
associated with a higher frequency of graft rejection and
leukemia relapses (Gale and Butturini (1988) Bone Marrow
15 Transplant 3:185; Devergie et al. (1990) ibid 5:379;
Filipovich et al (1987) Transplantation 44). Therefore,
the donor T cells appear to aid engraftment and to
provide a graft-versus-leukemia effect as well as causing
GVHD. Because the T cell burden following bone marrow
20 transplantation is low for the first 14 days (<10% or
normal) the log kill of donor T cells would be
proportionally enhanced (Marsh and Neville (1987) Ann.
N.Y. Acad. Sci. 507:165; Yan et al., submitted; Gale and
Butturini (1988) Bone Marrow Transplant 3:185; Devergie
25 et al. (1990) ibid 5:379; Filipovich et al. (1987)
Transplantation 44). It is expected that donor T cells
can be eliminated at set times during the early post
transplantation period using the present method. In this
way the useful attributes of grafted T cells might be
30 maximized and the harmful effects minimized.
    In a further embodiment, the present invention
relates to a method of treating autoimmune diseases in an
animal comprising administering to the animal anti-CD3-

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CRM9 or derivatives thereof under conditions such that the symptoms of the autoimmune disease improve.   In one preferred embodiment, the autoimmune disease is AIDS. Radiation induced T cell ablation with concomitant high 5 dose zidovudine therapy followed by bone marrow transplantation has been reported to eradicate HIV-1 infection in one case (Holland et al. (1989) Ann. Int. Med. 111:973).   Cyclophosphamide, a T cell suppressive reagent, has been shown to be beneficial in treating 10 murine AIDS (Simard and Joliceur (1991) Science 251:305). Anti-CD3-CRM9 can provide extensive T cell ablation without the requirement of bone marrow reconstitution.

Anti-T cell hemi-immunotoxins (mAbs conjugated to ricin A chain) have been used clinically as T cell 15 suppressants for the treatment of GVHD, rheumatoid arthritis and T cell leukemia (Program and Abstracts 2nd Int. Symposium on Immunotoxins, June 1990, Lake Buena Vista, Florida; Byers et al. (1990) Blood 75:1426). Some positive effects have been noted.   The immunotoxin 20 described here is more toxic on a weight basis than hemi-immunotoxins, but at tolerated doses exhibits an apparent 3 log kill of targeted cells at target cell burdens encountered clinically.   This constitutes a favorable therapeutic margin.   Most human sera contain anti-DT 25 neutralizing antibodies from childhood immunization (Johnson et al. (1989) J. Neurosurg. 70-240).   To compensate for this the therapeutic dose of anti-CD3-CRM9 can be appropriately raised without affecting the therapeutic margin. Alternatively, CRM197 a non-toxic DT 30 mutant reactive with neutralizing antisera, could be administered prior to the conjugate.

The present invention will be illustrated in further detail in the following non-limiting examples.

10

EXAMPLE 1
Establishment of Tumors

The experimental design of the studies that give
rise to the present invention was dictated by the goal of
5 having an animal model as closely relevant to human _in
vivo_ tumor therapy as possible. In order to minimize the
host killer cell immune responses, bg/nu/xid strain of
nude mice were used (Kamel-Reid and Dick (1988) Science
242:1706). The human T cell leukemia cell line, Jurkat,
10 was chosen because of previous studies with this line and
its relatively normal average complement of CD3 receptors
(Preijers et al. (1988) Scand. J. Immunol. 27:553). The
line was not cloned so that receptor variation among
individual cells existed (Figure 1 legend). A scheme was
15 developed whereby well established tumors of constant
mass equal to 0..1% of body weight ($\sim$4X10^7 cells) could be
achieved 7 days after inculcation of Jurkat cells (see
Figure 1 and Dillman et al. (1988) Cancer Res. 15:5632).
This required prior irradiation and inoculation with
20 lethally irradiated helper feeder cells (see Figure 1 and
Dillman et al. (1988) Cancer Res. 15:5632).

EXAMPLE 2
Guinea Pig Studies

Immunotoxins toxicity studies were performed in
25 guinea pigs, an animal (like humans) with a high
sensitivity to diphtheria toxin (mice are highly
resistant to diphtheria toxin). Therapy of CRM9
conjugates was set at 1/2 the guinea pig minimum lethal
dose. In this study, minimum lethal dose (MLD) is
30 defined as the minimum tested dose which results in both
non-survivors and survivors over a 4 week evaluation
period. All animals survive when a MLD reduced by 0.5.
MLD was evaluated in guinea pigs 300-1000 g by

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subcutaneous injection. The following MLDs were found
listed as μg of toxin/kg body weight; DT, 0.15; CRM9, 30;
anti-CD5-DT (cleavable), 0.65; anti-CD5-CRM9 (non-
cleavable), 150. Finally, the therapeutic efficacy of
5   the immunotoxin treatment in producing tumor regressions
was compared to graded doses of whole body irradiation
which resulted in similar tumor regressions.

EXAMPLE 3

Comparison of Immunotoxins

10   Several types of immunotoxins were compared is this
study. They were synthesized as previously described by
thiolating both the monoclonal antibody moiety and the
toxin moiety and then crosslinking the bismaleimide
crosslinkers (Neville et al. (1989) J. Biol. Chem.
15 264:14653). Purification was performed by size exclusion
HPLC columns and fractions containing 1:1 toxin:antibody
mol ratios were isolated for these studies. Conjugates
made with an acid-labile crosslinker bismaleimidoethoxy
propane were compared with a non-cleavable, bis-
20 maleimidohexane.   Conjugates made with this cleavable
crosslinker have been shown to hydrolyze within the
acidifying endosome releasing free toxin moieties with
half-times of hydrolysis measured at pH 5.5 of 36 mins
(Neville et al. (1989) J. Biol. Chem. 264:14653).

25   The results of this study are tabulated in Table I.
Non-treatment groups such as group 10, groups treated
with anti-CD5 immunotoxins (groups 5 and 6), and group 4
treated with a mixture of anti-CD3 and CRM9 did not show
regression. The vascularized tumor nodules that weighed
30 20 mg on day 7 grew to between 1.5 to 7.8 g on day 37 and
weighed between 7.9 and 11.6 on day 56.   No late
spontaneous regressions were noted. In contrast, group
1 consisting of treatment with anti-CD3-CRM non-cleavable

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conjugate (NC) given at 25 μg/kg on days 7, 8 and 9 (see Figure 1 time line) showed only 1 tumor out of 6 by day 37. Some of the remaining animals were subject to autopsy and they failed to reveal residual tumor or even

5 scaring. Tumors identified as regressed on day 37 by superficial inspection did not appear during the course of the study (56 days).

TABLE 1

Immunotoxin and Radiation Treatment on Subcutaneous

Human T Cell Tumors (Jurkat) in Nude Mice

Group	Treatment	Dose (intraperitoneal)	Animals Bearing Tumors At Day 37/Group Animals	% Tumors Regressions
1	Anti-CD3-CRM9 (NC)[a]	25 μg/kg. x 3d	1/6	83
2	Anti-CD3-CRM9 (NC)	19 μg/kg. x 2d	1/4	75
	Anti-CD5-CRM9 (C)	19 μg/kg. x 2d		
3	Anti-CD3-CRM9 (C)	25 μg/kg. x 3d	2/4	50
4	Anti-CD3+CRM9	25 μg/kg. x 3d	4/4	0
5	Anti-CD5-CRM9 (C)	25 μg/kg. x 3d	5/5	0
6	Anti-CD5-DT (NC)	25 μg/kg. x 1d	9/9	0
7	γradiation ^{137}Cs	400 cGy	2/2	0
8	γradiation ^{137}Cs	500 cGy	3/6	50
9	γradiation ^{137}Cs	600 cGy	0/2[b]	100
10	None		6/6	0

a. Anti-CD3 refers to the monoclonal antibody UCHT1 and was purchased from Oxoid USA, Inc. Anti-CD5 refers to the monoclonal antibody T101 and was a gift from Hybritech (San Diego). NC and C refer, respectively, to non-cleavable and cleavable conjugates.

b. These animals were evaluated on days 10 and 13 at the time of death from radiation sickness.

The cleavable crosslinker confers no therapeutic advantage to anti-CD3-CRM9 immunotoxins and may be less effective (group 3). Cleavable crosslinkers confer some advantages with anti-CD5-CRM9 conjugate in vitro (5) but
5 had no effect in this in vivo system (group 5), and lacked significant potentiating effect when administered with anti-CD3-CRM9 (group 2). The clevable crosslinker conferred a marked therapeutic crosslinker to anti-CD5 wild type toxin conjugates and tumor regressions were
10 achieved. However, in these cases the guinea pig toxic dose was exceeded. A single dose on day 7 of cleavable anti-CD5-DT at 6 µg/kg produced 8/10 tumor regressions while a cleavable conjugate made with an irrelevant antibody (OX8) produced no regressions (4/4). However,
15 this dose exceeded the guinea pig MLD by 9 fold. A rescue strategy was tried in which the above conjugate dose was given intravascularly followed by DT antitoxin 4 hours later (also intravascularly). The 4 hr. rescue could not raise the MLD above 0.65 µg/kg. The 1 hr.
20 rescue could not raise the MLD above 0.65 µg/kg. The 1 hr rescue raised the MLD to 36 µg/kg, however, there were no tumor regressions in 10 mice receiving 21.5 µg/kg of the cleavable anti-CD5-DT conjugate.

In groups 7-9 increasing single doses of whole by
25 radiation (102 cGy/min) were given to animals bearing 3X3X5 mm tumors. At 400 cGY no complete regressions occurred. At 500 cGy 50% complete tumor regressions occurred. At 600 cGy 100% regression was achieved as judged on day 10 and 13 when the animals died from
30 radiation sickness. (Groups 7-9 did not receive prior radiation and tumor takes were less than 100%). It appears that the 75 µg/kg anti-CD3-CRM9 (NC) immunotoxin is equal in therapeutic power to between 500 and 600 cGy

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of radiation.

EXAMPLE 4

Estimation of Cell Kill

The actual cell kill achieved by the radiation and
5 the immunotoxin can be estimated by assuming radiation
single hit inactivation kinetics along with a D_{37} value
for the radiation. A value for D_{37} of 70-80 cGy with n =
1.2-3.0 is not unreasonable for a rapidly dividing helper
T cell. D_{37} is the dose of radiation which reduces the
10 fraction of surviving cells to 1/e as extrapolated from
the linear portion of the log survivors vs. dose curve
and n is the intercept at 0 dose (Anderson and Warner
(1976) in Adv. Immunol., Academic Press Inc., 24:257).
At a dose of 550 cGy the fraction of surviving cells is
15 calculated to be about 10^{-3}. Since a majority of tumors
completely regress at this dose we estimate that both
therapies are producing an approximate 3 log kill. (The
remaining cells, $4X10^{7}X10^{-3} = 4X10^{4}$ cells cannot apparently
maintain the tumor, i.e., the in vivo plating efficiency
20 is low, a fairly typical situation in the nude mouse
xenograft system.) The reliability of this 3 log kill
estimate has been verified by determining the tissue
culture plating efficiency by limiting dilution of 7 day
established Jurkat tumors (following dispersal) and
25 tumors exposed 18 hours earlier in vivo to 600 cGy.
Plating efficiencies were 0.14 and $1.4X10^{-4}$, respectively.
(Plating efficiency is the reciprocal of the minimum
average number of cells per well which will grow to form
one colony.

30 It should be emphasized that with high affinity
holo-immunotoxins the cell kill is inversely proportional
to the target cell number. This presumably occurs
because receptors are undersaturated at tolerated doses

and free conjugate concentration falls with increasing
target cell burden (Marsh and Neville (1987) Ann. N.Y.
Acad. Sci. 507:165; Yan et al., submitted) To Put this in
perspective, the tumor burden in this study is almost
5   equal to the number of T cells in a mouse ($\sim 10^8$). If the
extrapolation to humans holds, it could be expected that
a tolerated dose of ant-CD3-CRM9 immunotoxin could
achieve an _in vivo_ 3 log depletion of a normal number of
CD3 positive T cells.

10   *   *   *   *   *   *

All publications mentioned hereinabove are hereby
incorporated in their entirety by reference.

While the foregoing invention has been described in
some detail for purposes of clarity and understanding, it
15 will be appreciated by one skilled in the art from a
reading of this disclosure that various changes in form
and detail can be made without departing from the true
scope of the invention and appended claims.

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WHAT IS CLAIMED IS:

      1.   An immunotoxin comprising anti-CD3-CRM9 or derivatives thereof.

      2.   The immunotoxin of claim 1, wherein said derivative contains an altered CRM9 C-terminus and a diminished capability of binding to non-target cells.

      3.   The immunotoxin of claim 1, wherein said anti-CD3-CRM9 or derivatives thereof contain a non-cleavable crosslinker.

      4.   The immunotoxin of claim 3, wherein said crosslinker is bismaleimidohexane or m-maleimidobenzoyl-N-hydroxysuccinimide ester.

      5.   A pharmaceutical composition comprising anti-CD3-CRM9 or derivatives thereof in an amount effective to treat T cell leukemias or lymphomas which carry a CD3 epitope, graft-versus-host disease or autoimmune diseases, and a pharmaceutically acceptable diluent, carrier, or excipient.

      6.   The pharmaceutical composition according to claim 5, wherein said derivative contains an altered CRM9 C-terminus and a diminished capability of binding to non-target cells.

      7.   Use of anti-CD3-CRM9 or derivatives thereof in the treatment of T cell leukemias or lymphomas which carry a CD3 epitope in an animal wherein anti-CD3-CRM9 or derivatives thereof are administered to said animal under conditions such that said leukemias or lymphomas regress.

      8.   Use of anti-CD3-CRM9 or derivatives thereof in the treatment of graft-versus-host disease in an animal wherein anti-CD3-CRM9 or derivatives thereof are administered to said animal under conditions such that the symptoms of said graft-versus-host disease improve.

9.   Use of anti-CD3-CRM9 treatment of autoimmune diseases in an animal wherein anti-CD3-CRM9 or derivatives thereof are administered to said animal under conditions such that the symptoms of said autoimmune
5   disease improve.

10.   Use according to claim 9, wherein the autoimmune disease in AIDS.
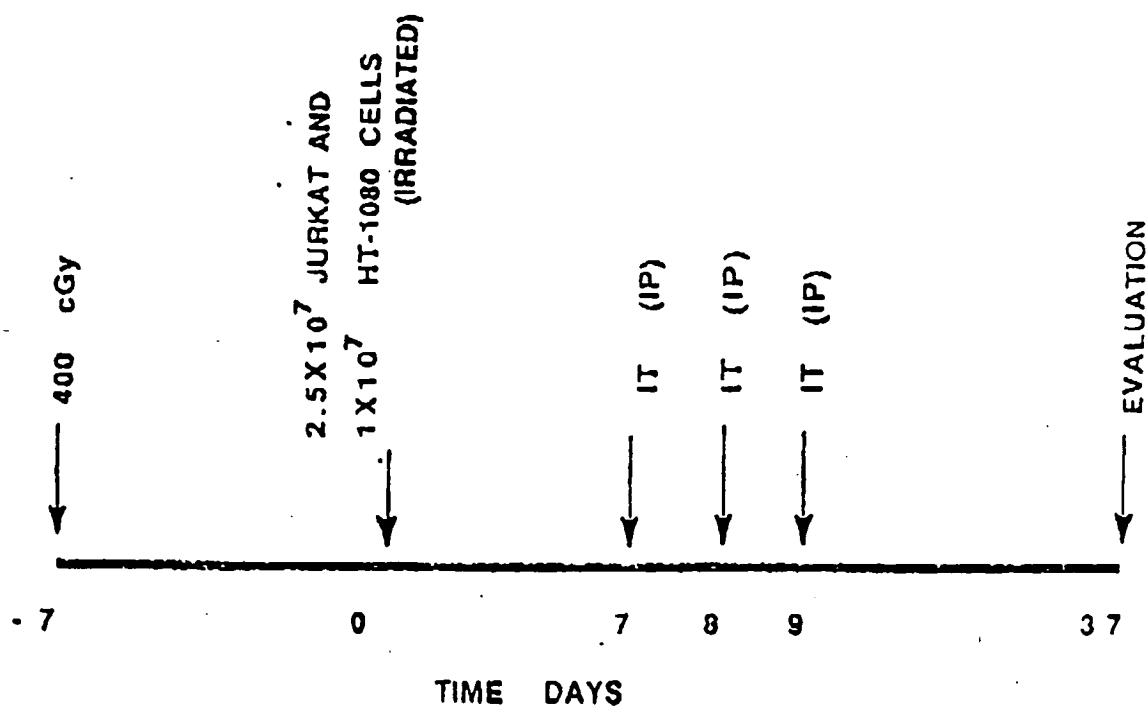
FIGURE 1

INTERNATIONAL SEARCH REPORT

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): A61K 39/44; C07K 15/28, 17/02
US CL : 424/85.91; 530/391.7, 391.9

II. FIELDS SEARCHED

Minimum Documentation Searched[4]

Classification System	Classification Symbols
U.S.	424/85.91; 530/391.7, 391.9

Documentation Searched other than Minimum Documentation
to the extent that such Documents are included in the Fields Searched[5]

Please See Attached Sheet.

III. DOCUMENTS CONSIDERED TO BE RELEVANT[14]

Category[*]	Citation of Document,[16] with indication, where appropriate, of the relevant passages[17]	Relevant to Claim No.[18]
x/y	Journal of Biological chemistry, Volume 264, number 25, issued 05 September 1989, D. M. Neville, Jr. et al, "Enhancement of Immunotoxin Efficacy by Acid-cleavable Crosslinking Agents Utilizing Diphtheria Toxin and Toxin Mutants", pages 14653-14661, see pages 14653(abstract), 14654, 14657(Table II), 14658 and 14660.	1, 2-5/1-9
y	Science, volume 238, issued 23 October 1987, L. Greenfield et al, "Mutations in Diphtheria Toxin Separate Binding from Entry and Amplify Immunotoxin Selectivity", pages 536-539, see the abstract at page 536.	1-6
y	Journal of Immunological Methods, Volume 121, issued 1989, D. E. Myers et al, "The effects of aromatic and aliphatic maleimide crosslinkers on anti-CD5 ricin immunotoxins", pages 129-142, see pages 129(abstract) and 131.	1-6
y	EP, A, 0 306 943 (Sivam) 15 March 1989. See the abstract and Example V at pages 11-12.	1-6

* Special categories of cited documents:[16]
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
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IV. CERTIFICATION

Date of the Actual Completion of the International Search[2]	Date of Mailing of this International Search Report[2]
27 APRIL 1992	1 2 MAY 1992
International Searching Authority[1]	Signature of Authorized Officer[20]
ISA/US	Kay K. Kim, Ph. D.

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FURTHER INFORMATION CONTINUED FROM PREVIOUS SHEETS

II. FIELDS SEARCHED
Other Documents Searched:

CAS ONLINE, APS
Search terms: antibody, antibodies, immunoglobulin, immunoglobulins, link, linking, linked, crosslinking, crosslinked, conjugate, conjugates, conjugated, conjugation, conjugating, toxin, toxins, anti-CD3-CRM, anti-CD3-CRM9, diphtheria, bismaleimidohexane, maleimidobenzoyl and hydroxysuccinimide